

INTRINSIC VISCOSITY DETERMINATION BY “SINGLE-POINT” AND “DOUBLE-POINT” EQUATIONS

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ABSTRACT:

“Single-point” equations used for intrinsic viscosity determination are greatly used when working with synthetic polymer solution systems. In this work we have applied them to a biological macromolecule in a bovine serum albumin (BSA)/water system. Almost all single-point equations are available and errors can be lowered. However, we have detected a systematic bias in the estimations provided by “single-point” methods. To overcome it we propose a “double-point” method which gives lower estimation errors for this system. This novel method is not system specific and could be applied to other polymeric solution.

ZUSAMMENFASSUNG:

Single-Point-Gleichungen (Einzelpunkt-Gleichungen) zur Bestimmung der inneren Viskosität werden in hohem Maße bei der Arbeit mit Systemen synthetischer Polymerlösungen angewandt. In dieser Arbeit haben wir sie auf ein biologisches Makromolekül eines Rinder-Serum-Albumin (BSA)/Wasser-Systems angewandt. Fast alle Single-Point-Gleichungen stehen zur Verfügung und die Fehlerquoten können niedrig gehalten werden. Wir haben jedoch eine systematische Abweichung in den Schätzungen, welche die Single-Point-Methode liefert, vorgefunden. Um diese zu überwinden, schlagen wir eine Double-Point-Methode (Zweipunkte-Methode) vor, die niedrigere Schätzwerte für dieses System erlaubt. Diese neue Methode ist nicht systemspezifisch und kann auf andere Polymerlösungen angewandt werden.

RÉSUMÉ:

Les équations de simple point pour la détermination de la viscosité intrinsèque sont très employées en systèmes de solutions de polymères synthétiques. Dans ce travail-ci nous les avons appliquées au système: eau-BSA (macromolécule biologique), et, nous y avons détecté un erreur systématique dans les estimations données par cette méthode. Dans ce travail, on montre graphiquement l'erreur que on peut obtenir. Pour résoudre cette difficulté on présente ici une équation pour l'estimation en utilisant une méthode de double point, d'application générale et qui diminue l'erreur commise. Cette nouvelle méthode n'est pas spécifique à ce système et elle peut être appliquée à les autres polymères en solution.

KEY WORDS: intrinsic viscosity, “single-point”, “double-point”, BSA

1 INTRODUCTION TO TECHNICAL FIBRE SUSPENSIONS

With rheology progress was made in understanding of structure of gels and colloidal suspensions, particularly with regard to polysaccharides and large glyconjugates. Whereas the rheology of concentrated dispersions is the considerable interest to the colloidal scientist, pharmacist or food technologist [1], of more interest to molecular biophysicist is dilute solution viscometry and the structural parameter the intrinsic viscosity [2]. The intrinsic viscosity is a viscos-

ity measure extrapolated to infinite dilution that depends on the properties of isolated macromolecules in solution (effects of interaction have been eliminated by extrapolation). So, intrinsic viscosity provides information about molecular weight, radius of gyration, shape, specific volume, conformation, hydration, flexibility of the macromolecules. Several empirical equations relating viscosity to concentration [2 - 4] have been proposed in order to determine intrinsic viscosity $[\eta]$. The three most commonly used equations, concerning dilute solutions, are the following:

obtained at $C = 0.5$ as well. In the particular case of $C = 0.125$ where we do not have available data at a lower concentration, the reported estimation is that of Ram-Mohan-Rao and Yassen [11].

From the graphics we can see that the “double-point” method has a very consistent behavior when compared with the “Multiple Linear Least-Squares Fits with a Common Intercept method”, although is even better in the case of the system dealt with in Figure 3. In the last column of Tables 2 to 6 we have included the percentage relative errors of the estimations obtained by our ‘two point’ method given in Equation 12 and where the observations made in the previous paragraph have been taken into account.

4 CONCLUSION

Although the suitability of ‘single-point’ equations has been objected, some automated viscosimeters have already incorporated them into their software. Also, there is a continued interest in verifying their applicability to different systems [15]. However, we have not found any reference towards biological macromolecule solutions. In this work we have studied the BSA-water system and pH variation. BSA is a non-linear, ramified, highly cross-linked molecule with strong internal and water interactions, in which it is soluble. BSA is globular, heart shaped at neutral pH [20] and has more extended conformations as pH changes. The intrinsic viscosity of BSA solutions changes at different pH because BSA size and shape are modified. Regarding diluted BSA solutions, viscosity dependence on concentration is unusual [17, 21].

The highest errors are observed in the run at pH 8.06, with data presenting a linear regression (R^2) of 0.983, but with a negative and very high K_H . Also, the sum of the K_H and K_K constants is more deviated from the 0.5 value. At the remaining runs, $K_H + K_K$ deviates approximately 25 % and error decreases 50 %. All runs have values of $\eta_i < 0.1$ [10] but the obtained values were acceptable. All equations show dependence on concentration and enhance their outcome at concentrations lower than 1 %, except for Equation 10 that shows a non-systematic behavior. $K_{SB} = 0.28$ was used in Equation 11 [15] although a very different value is observed in Table 1. In general, it can be stated that these equations (except Equation 10) can be used for approximate determinations of intrinsic viscosity in the system BSA-water when concentra-

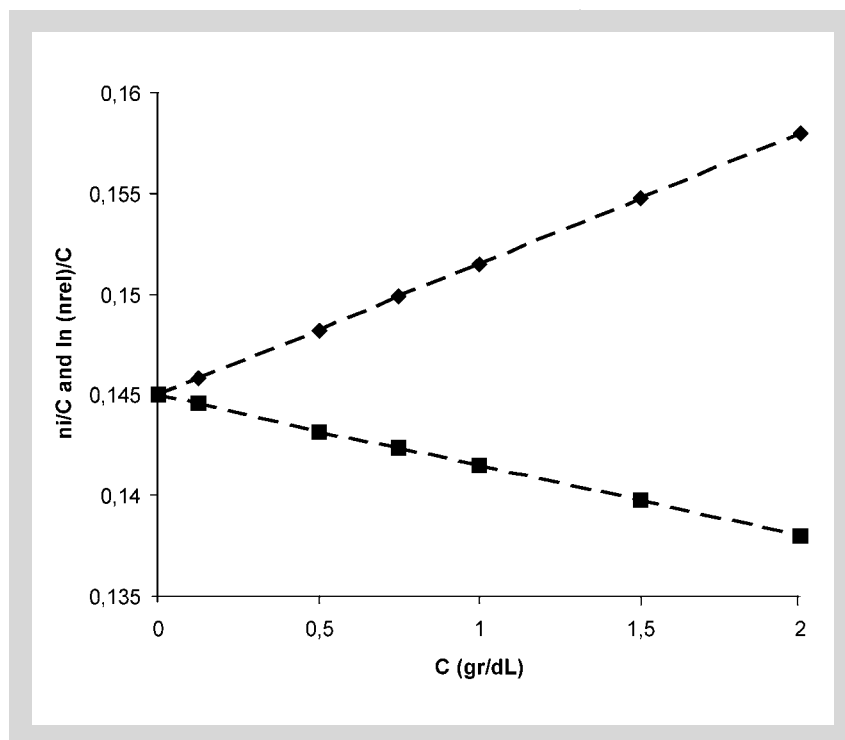


Figure 2: Graphic representation (Huggins and Kraemer's equations) of the results of viscosimetric measures of a solute-solvent system for which 'single-point' estimators provides good estimations for $[\eta]$.

tions are inferior to 0.75 %. For higher concentrations, all “single-point” estimators provide poor estimations, and a graphical explanation for this behavior is provided. Therefore, we propose a “double-point” method to estimate $[\eta]$ which have given errors smaller than 3 % in all the system studied in this paper, except for the case pH = 8.6 where an error of 11 % is observed, although it may be due to two experimental points which seem to be no coherent [17]. Our method not require a graphical adjustment and is more economic than that presented in [19], which simultaneously adjust series of Huggins and Kraemer's values with two lines with a common intercept at $C = 0$, providing good estimates for $[\eta]$, but requiring of at least four determinations.

Methods based upon “single point” equations are strongly dependent on the system under consideration, its concentration and the verification of the condition $K_H + K_K = 0.5$. On the other hand, regarding the BSA-water system (which presents different rheological behaviors), the “double-point” method of estimation performs better, exhibits less dependence on the concentration and does not require of previous knowledge about the values of K_H and K_K .

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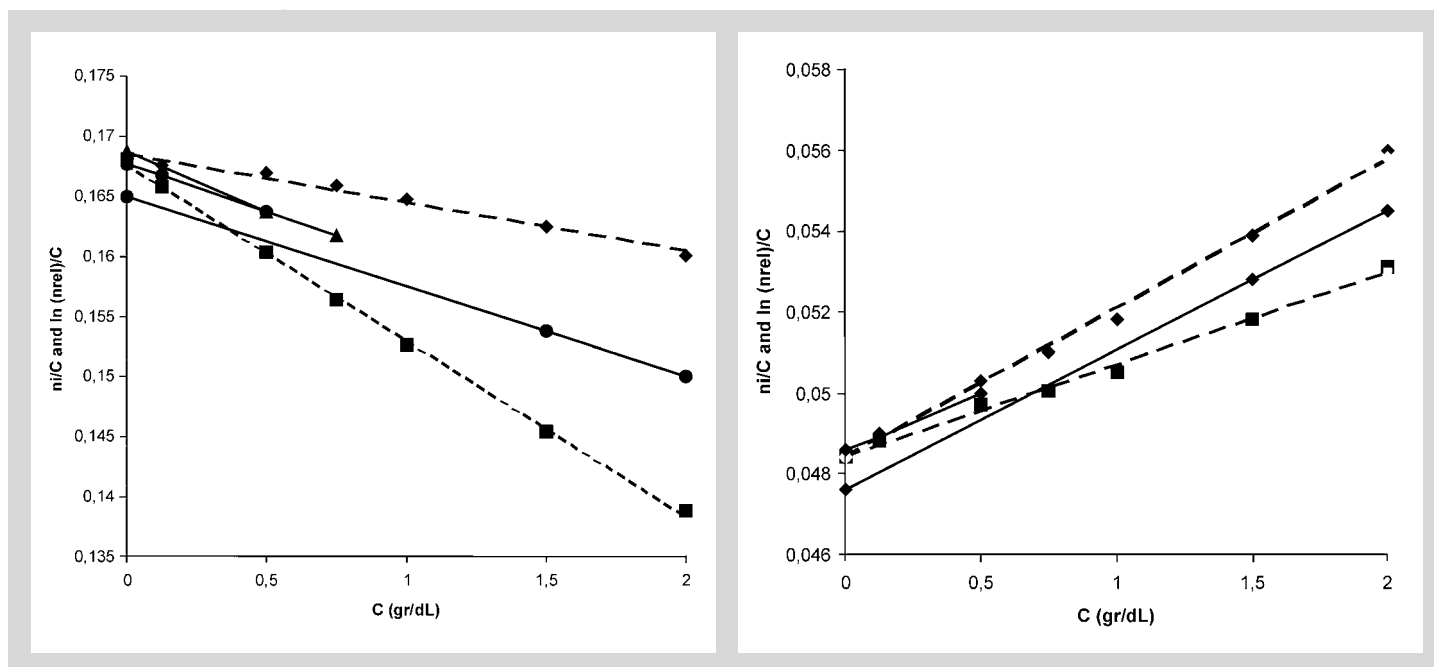


Figure 3 (left): 'Double-point' estimation of $[\eta]$ for the BSA-water system at pH = 2.71 (Dotted line: Multiple Linear Least-Squares Fits with a Common Intercept method; Solid lines: Lines joining two consecutive Ram-Moham-Rao and Yassen estimations (represented by the solid squares in the graphic)). The double-point estimation is the point of each solid line just on the vertical axis.

Figure 4: 'Two-point' estimation of $[\eta]$ for the BSA-water system at pH = 7.4 (Dotted line: Multiple Linear Least-Squares Fits with a Common Intercept method; Solid lines: Lines joining two consecutive Ram-Moham-Rao and Yassen estimations (represented by the solid squares in the graphic)). The double-point estimation is the point of each solid line just on the vertical axis.

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